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The objective of this project is to use primarily an electrochemical approach to study the fundamental molecular processes that underlie the light-mediated sensory (visual) and energy (photosynthetic) transduction in model retinal protein membranes. We study the fast photoelectric signal which appears both in the visual photoreceptor membrane as well as in model membranes reconstituted from bacteriorhodopsin or halorhodopsin. We experimentally distinguished three reporter signals from the hydrophobic chromophore binding pocket region (B1 component) and from the hydrophilic domains of the two membrane surfaces (B2 and B2' components). We developed a concept of local reaction conditions which is applicable to all pigment-containing thin films or membranes. We analyzed the photosignals in the framework of intelligent materials. A similar photosignal was discovered in a reconstituted halorhodopsin membrane. Preliminary data from mutant bacteriorhodopsins synthesized by site-directed mutagenesis are also reported. An electrostatic mechanism of visual transduction mechanism is proposed. Our analysis suggests that there is a common mechanistic design among these membranes.

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FINAL REPORT ON CONTRACT N00014-87-K-0047

PRINCIPAL INVESTIGATOR: Felix T. Hong

CONTRACTOR: Wayne State University

CONTRACT TITLE: Electrochemical Study of Phototransduction in Protein Pigment-Containing Model Membranes

START DATE: 1 December 1986

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RESEARCH OBJECTIVE: The objective is to use an electrochemical approach to study the fundamental molecular processes that under lie the physical basis of sensory transduction and energy transduction in retinal protein membranes.

RESEARCH PROGRESS:

Introduction:

Most light-mediated energy converting membranes utilize chlorophyll protein complexes as the reaction centers for photon energy conversion whereas most light-mediated sensory transducing membranes utilize retinal proteins as the light-sensing elements. The only thing in common to both types of membranes appears to be the asymmetrical orientation of the membrane-bound pigment-proteins. The discovery of bacteriorhodopsin in the purple membranes of *Halobacterium halobium* offers a new perspective which suggests that there may be a common design between the light energy transducing membranes (photosynthesis) and the photosensory transducing membranes (vision). This is because bacteriorhodopsin resembles the visual pigment chemically but functions as a photosynthetic pigment. Like the visual pigment rhodopsin, bacteriorhodopsin contains vitamin A aldehyde as the chromophore and both pigments belong to a class of membrane-bound proteins with an increasing list of members, the retinal proteins. Furthermore, there are three additional retinal proteins in *Halobacterium halobium*. While bacteriorhodopsin is a light-driven proton pump, halorhodopsin which exists in the red membrane of *Halobacterium halobium* is a light-driven chloride ion pump. Sensory rhodopsin I and II, also found in the red membrane, function as sensory pigments for phototaxis. These pigments share considerable sequence homology in the chromophore binding pocket. The study of these pigments (rhodopsinology) thus offers unprecedented opportunity to unravel the basic design principles on both the light energy converting pigments and the light signal transducing pigments from both a mechanistical and evolutionary point of view.

The function of these retinal proteins depends on the integrity of a supporting matrix, the membrane. Electrochemical analysis is indispensable. We have developed an electrochemical technique that allows the experimentalist to unambiguously separate photoelectric signals from different domains of the

pigment, i.e., our technique enables us to obtain site-specific photokinetic information. In conjunction with a newly developed expression system using *Halobacterium halobium*, which provides an efficient system for genetically engineering the retinal proteins, we began to obtain data that permit unambiguous correlation of site-directed structural changes to site-specific functional changes. We also began to generate meaningful conclusions as to whether Nature might have utilized a common design in photosynthetic and visual pigments. A concise account of our present accomplishments is provided below.

Detailed Progress:

(1) Concept of local reaction conditions and site-specific photoelectrokinetics in a pigment-containing membrane and the "differential" experiment:

We have previously concluded that the light-induced charge separation concurrent with the chromophore isomerization in bacteriorhodopsin can generate a fast photosignal (which we named the B1 component). In addition, the rapid processes of proton uptake at the cytoplasmic surface and proton release at the extracellular surface can also generate two separate photosignals, which we named the B2 and the B2' component. Our analysis has established that the B1 component is pH independent, but the B2 and the B2' component are pH-sensitive. Using a reconstitution method previously developed by Trissl and Montal (Nature, 266:655-657, 1977), the B2' component can be eliminated because the extracellular surface of bacteriorhodopsin is attached to a thin Teflon substrate and therefore the proton release there is suppressed. We developed a second method, the multilayer thin film method, to further suppress the B2 component and isolate the B1 component in pure form (Okajima and Hong, Biophys. J. 50:901-912, 1986). Our component analysis based on this scheme provides the only consistent methodology to analyze the fast photosignal from bacteriorhodopsin membranes. Alternative approaches inevitably led to striking discrepancies between data reported by various laboratories. Previously, we elucidated the reason for the discrepancies of using an electrochemical approach (Okajima and Hong, Biophys. J. 50:901-912, 1986).

The present study provided the first experimental identification of the existence of the B2' component. The separation of these two components is not straightforward and requires an electrochemical approach. Our effort to achieve this objective leads to the formulation of the concept of local reaction conditions, which is generally applicable to other photobiological membranes. The concept predicted that the B2' component will depend on the pH of the extracellular side but not the intracellular side. The "differential" experiment shown in Fig. 1 clearly demonstrated that the B2' component depends the pH of the extracellular space and that the pH dependence is opposite to that of the B2, thus allowing for unambiguous separation. The detail is found in publication A1 (Hong, J. Electrochem. Soc. 134:3044-3052, 1987).

Together with the experimental proof of the separation of the B1 from the concurrent B2 component (to be described in the next section), we have established an electrochemical analysis of the bacteriorhodopsin membrane which provides three site-specific photoelectric signal components originating

from the intracellular domain (B2), the chromophore binding pocket (B1) and the extracellular surface (B2'). This approach is summarized in publication A8 (Hong, in Protein Engineering: Protein Design in Basic Research, Medicine, and Industry, Edited by M. Ikehara et al, pp. 235-242, Japan Scientific Press/Springer Verlag, 1990).

(2) The "Q-tip" experiment and the experimental basis of our unique component analysis:

The experimental separation of the B1 from the B2 component is based on two reconstitution methods and on electrochemical analysis based on the Gouy Chapman's theory. The experimental basis of the methodology is established by the "Q-tip" experiment to be described below. The basis of this experiment is that the B1 component being generated from within the pigment does not require the presence of water. In contrast, the B2 component is generated from the cytoplasmic domain of the pigment and requires the access of aqueous protons for its generation. The working hypothesis is that the B1 component is additive in an oriented multiple layer thin film, but the B2 component can only be generated from the top layer (Fig. 2). Thus, the pH dependence which is attributed to the B2 component is diminished in a multiple layer thin film. The more prominent pH dependence seen in a Trissl-Montal film can be restored by removing all but the innermost layer by stripping the surface with a Q-tip (Fig. 3A & 3B). This happens only in a freshly prepared multilayer thin film. In contrast, in a film that has been aged for more than four days, the pH dependence disappears completely (Fig. 4A). Stripping with a Q-tip merely reduces the signal amplitude but does not restore the pH dependence (Fig. 4B). This demonstrated that the exposed hydrophilic domain is vulnerable to denaturation by drying in the air whereas the chromophore pocket, being protected from direct exposure to the outside environment is much more stable. Thus, the B2 component can be eliminated by prolonged drying. The B1 component persists in a rehydrated thin film after drying in the air for as long as three months.

This demonstrated the importance of our component analysis which is based on the generation mechanisms of the individual components and an experimental methodology that is consistent with the underlaying mechanism. We have found that the experimental methodology can also be applied to reconstituted halorhodopsin thin film. The experiment is summarized in publication C6 (Michaile and Hong, Proc. IEEE/EMBS, 11:1333-1335, 1989). A full length paper is in preparation.

(3) Analysis of halorhodopsin membranes:

Our theoretical analysis based on the Gouy Chapman method indicates that whenever there is a light-induced charge transfer across the membrane-water interface, there will be a fast photoelectric signal similar to the B2 and the B2' component. Since halorhodopsin transports chloride ions, we expect to see a B2 like signal that is sensitive to Cl^- rather than H^+. That this is indeed the case is shown in Fig. 5. The detail can be found in publication C8 (Mi-

chaiel et al., Proc. IEEE/EMBS, 12:1721-1723, 1990).

(4) Electrochemical analysis of mutant bacteriorhodopsins:

We have demonstrated the predictive power of our electrochemical methodology. We further enhance its power by combining it with recombinant DNA technology. Previously, mutant bacteriorhodopsins could only be obtained either by spontaneous mutation or by means of an expression system using *E. coli* (Dunn et al., J. Biol. Chem. 262:9264-9270, 1987). The latter method is inefficient in transformation and labor-intensive. An extensive reconstitution procedure is required, leading to unstable mutant pigments in low yield. Furthermore, the published photoelectric behaviors of these mutants shared the same problem with the data of wild type bacteriorhodopsin as indicated in section 1.

These difficulties have been overcome with a new expression system utilizing *Halobacterium halobium* developed by my collaborator Richard Needleman in the Biochemistry Department (Ni et al., Gene, 90:169-172, 1990). The new method offers large quantities of mutant bacteriorhodopsins with exceptional stability. We have begun to generate meaningful photoelectric data. Shown in Fig. 6 is the pH dependence of reconstituted wild type BR and mutant (212 Aspartate → Asparagine). The result shows that the B2 component is completely absent in the range of pH 4-11. At the low pH range, the B2 component appears to reverse its direction. Furthermore, this component is more prominent in 4M NaCl. By replacing chloride ions with sulfate ions, we demonstrated that the B2 component is completely absent even at low pH. Addition of less than a mM of NaCl brings this signal back. The presence of a high concentration of sulfate ions rules out the effect of the ionic strength, or charge screening or the presence of Na^+. We concluded that the signal is attributed to chloride ions.

Our preliminary data obtained from mutants 115 Aspartate → Asparagine, 96 Aspartate → Asparagine, and 85 Aspartate → Asparagine demonstrate that each mutant examined so far exhibits unique photoelectric behavior. The result is summarized in two manuscripts in preparation.

(5) Concept of Intelligent Materials:

The prediction of pH dependence of the B2 and the B2′ component was based on the law of mass action. Both B2 and the B2′ components can be affected by pH via the direct effect of changes of the concentration of one of the reactants. They can also be affected indirectly by the action of changing pH on the proton binding constant. The "differential" experiment shown in Fig. 1 indicates that the pH dependence is due to the latter effect. The physiological significance is best summarized by the concept of intelligent materials. The action of BR as a proton pump generates a transmembrane proton gradient which will exert a retarding force to reduce the efficiency of further pumping by virtue of the law of mass action. The observed pH dependence of the B2 and the B2′ component indicates that the pK_a of the extracellular

and intracellular binding sites is pH dependent so as to compensate for the reduction in efficiency. The detailed discussion can be found in publication A10 (Hong, in Biomedical Engineering in the 21st Century, Edited by C.-Y. Wang et al., pp. 77-84, Huei-Wen Science Publ., Taipei, 1990) . The idea is further expanded to include both the photosynthetic membranes and visual membranes and is summarized in publication A12 now in press (Hong, Nanobiology, Vol.1, No. 1, in press).

(6) Common design of photosynthetic and visual pigments:

The presence of a similar photoelectric signal in visual membranes, the early receptor potential (ERP), with analogous signal components R1 and R2 suggests that there may be a common design of photosynthetic and visual pigments. However, the ERP has long been dismissed as an epiphenomenon, an evolutionary vestige without any physiological function. We have previously argued from the electrochemical point of view that such a conclusion is premature (see Publication C2, Hong, Proc. IEEE/EMBS, 9:304-307, 1987).

Comparison of the photoelectric behavior of three retinal proteins, rhodopsin, bacteriorhodopsin, and halorhodopsin suggests that the R1, B1, and H1 component represents initial storage of photon energy in the form of charge separation. This energy is utilized subsequently to drive conformational changes of the respective protein to serve different functions. We reported evidence that the subsequent charge uptake and/or release at the membrane surfaces are light-assisted and are therefore a consequence of the conformational changes. In the case of bacteriorhodpsin and halorhodopsin, light-induced charge (protons or Cl^-) separation is the major event, which requires both charge uptake and release at opposite surfaces. In the case of rhodopsin, there is no need to convert energy by moving protons all the way across the membrane. The presence of proton uptake that leads to the generation of the R2 component is more subtle. Based on our electrochemical analysis, we proposed an electrostatic regulatory mechanism of visual phototransduction.

It is known that the activation of transducin (G-protein), which precedes photoexcitation, occurs at the stage of metarhodospin I to metarhodospin II transition of the rhodopsin photochemical relaxation. The latter reaction coincides with the R2 component of the ERP and with a rather significant surface potential change of the visual membrane. This surface potential change leads to a rapid change of interfacial concentration of physiologically important ions, and also exerts a rapidly varying internal electric field inside the membrane. It thus provides an ideal "switching-on" (triggering) mechanism of phototransduction in vision, because it is swift and highly localized. It is known that during the inactivation of phototransduction a significant number of threonine and serine residues near the C-terminus of rhodospin are phosphorylated. The resulting reduction of the surface potential at the cytosol side could provide a switching-off mechanism. Such a mechanistic model is viable only if the proton uptake accompanying the R2 component also occurs on the cytosol side of the membrane.

Evidence that the R2 component represents a molecular process at the

cytosol surface was provided by an experiment reported by M. A. Ostrovsky's group in Moscow State University (Shevtchenko et al. Sensory Systems USSR Acad. Sci. 1:117-126, 1987, in Russian). By measuring light-induced pH changes in both inside-out and right-side-out visual membrane vesicles, they concluded that the proton uptake indeed occurs at the cytosol side. They also found no proton release at the opposite (intradiscal) side. These findings together with the experimental detection of a light-induced surface potential reported by Cafiso and Hubbell (Biophys. J., 30:243-264, 1980) make the above-mentioned electrostatic regulatory mechanism of visual transduction eminently viable. Furthermore, the finding that there is only proton uptake at the cytosol side and no proton release at the intradiscal side is also consistent with the role of rhodopsin as a photon signal sensor rather than a photon energy converter. While further experimentation is needed to establish such a mechanism, the ERP can no longer be dismissed as an epiphenomenon.

We also compare the function of bacteriorhodopsin and that of the bacterial reaction center of *Rhodopseudomonas viridis*. In spite of the structural difference, the two photosynthetic apparatuses operate on the basis of similar principles in enhancing the forward charge transfer and in retarding the reverse charge transfer. This result together with the above analysis of visual transduction mechanism suggests that "reverse engineering" may be a viable approach in deciphering the secret of design principles which Nature utilized and perfected through billions years of evolution. The discussion is summarized in Publication A6 (Hong, J. Molec. Electronics, 5:163-185, 1989) and is further elaborated in Publication A11 (Hong, in Molecular Electronics and Biocomputers, Edited by P. I. Lazarev, pp. 291-310, Kluwer Academic Publ. Dordrecht, 1991).

CONCLUSIONS:

The objective of the Accelerated Research Initiative on Membrane Electrochemitry is to elucidate sensory and transport mechanisms and to unravel a possible common design mechanism. We select a class of membrane proteins, retinal proteins, for the investigation summarized in the present report. The present study shows that there is indeed a common design at the level of mechanistic operation. Our results further demonstrated that electrochemistry is an indispensable tool. Many seemingly incomprehensible phenomena eventually were provided with simple mechanistic explanations based on electrochemistry. The predictive power of our electrochemical analysis also allows us to take short cuts to arrive at important observations and to reach unequivocal conclusions in a cost-effective manner, both with regards to time and financial resources.

Our main conclusions are summarized in publication A14 (Hong, in Biomembrane Electrochemistry, ACS Advance in Chemistry Series, Edited by M. Blank and I. Vodyanoy, American Chemical Society, Washington, DC). The technological implications of our present work is presented in a number of articles related to molecular electronics (Publications A5, A7, A9, A13, C3, C4, C5, C7) and several drafts of reports to government workshops (Publications D1, D2, D3).

TRAINING ACTIVITIES:

Michaile, Sherie, Postdoctoral Research Associate.

Petrak, Michelle, Graduate Student.

Demographic Data: Women or minorities - 2; Citizens: 2

INVENTIONS: None

PATENTS: None

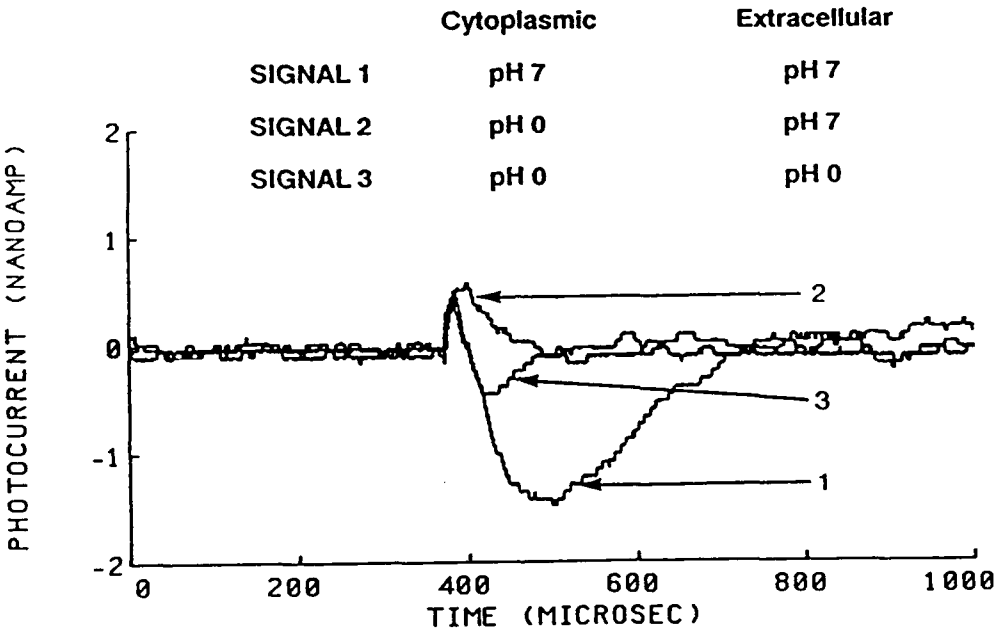

Fig. 1. The "differential" experiment showing the presence of two pH sensitive components, B2 and B2′. Both components have a negative polarity while B1 has a positive polarity. Signal 1 shows the presence of both B1 and B2. Signal 2 shows only B1. Signal 3 shows the presence of B1 and B2′. See text for explanation.

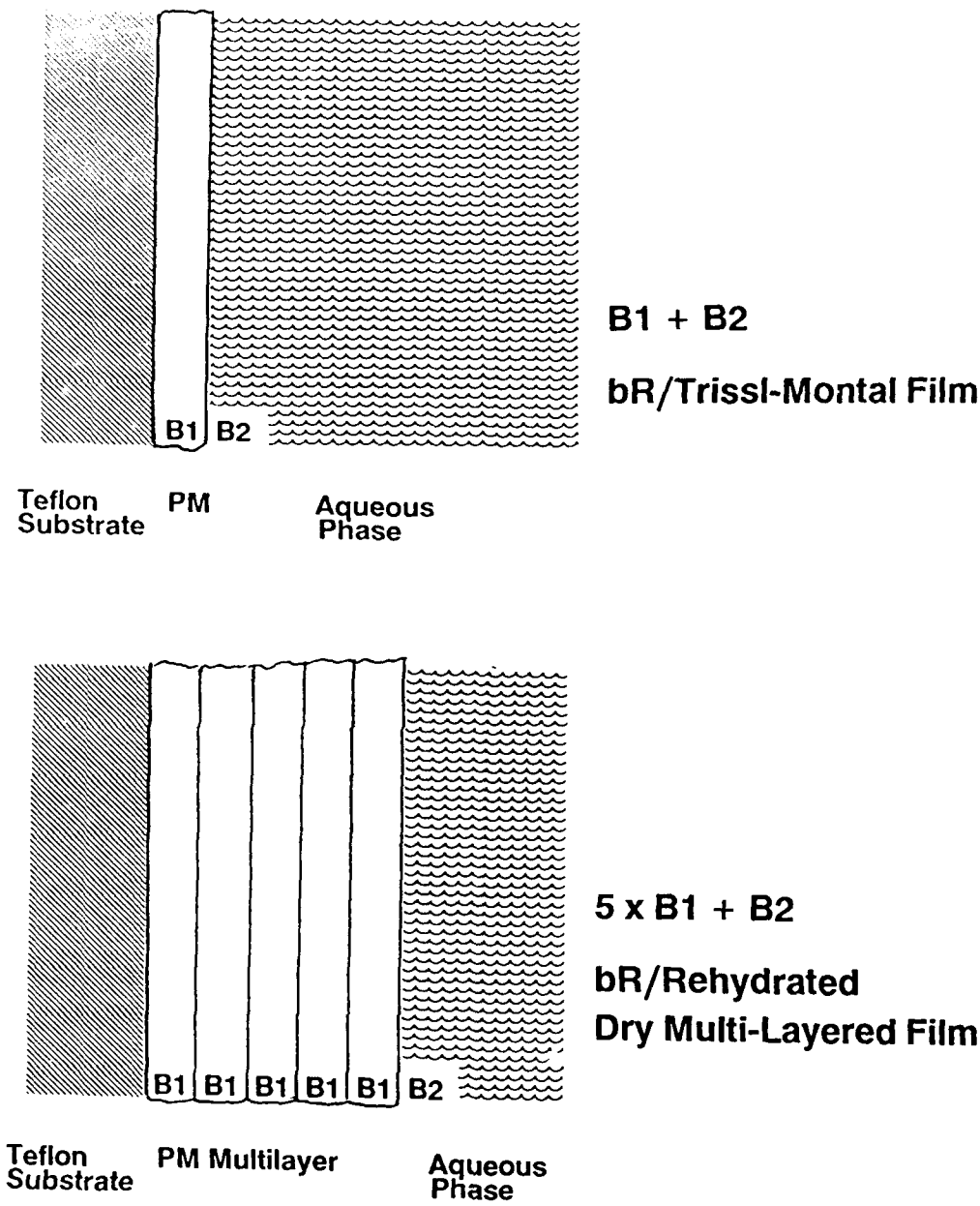

Fig. 2. Schematic showing the difference of a Trissl-Montal Film (A) and a rehydrated dry multi-layered film (B). See text for explanation.

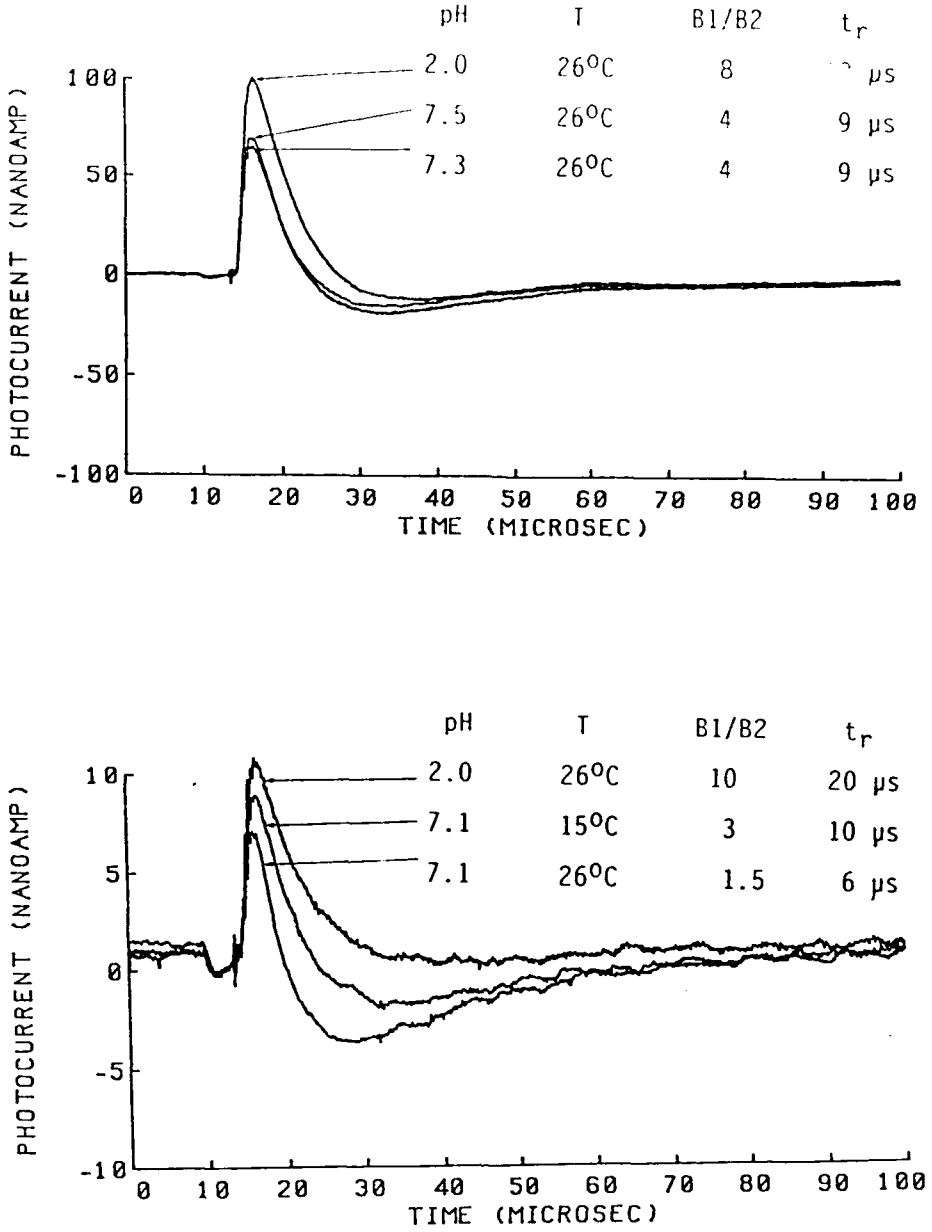

Fig. 3. (A) Effect of pH on the photoelectric signal from a fresh multi-lay ered bR film. The aqueous phase contained 0.1 M KCl and 0.01 M L-histidine buffered at the indicated pH. The temperature was 26°C. The data were taken in the following order: pH = 7.3, pH = 2.0, and pH = 7.5. The access impedance was 40 kΩ, and the instrumental time constant was 1.33 μs.

(B) Effect of pH and temperature on the photoelectric signal from a fresh multi-layered bR film after 'stripping'. The aqueous phase contained 0.1 M KCl and 0.01 M L-histidine buffered at the indicated pH. The data were taken in the following order: pH = 2 at 26°C, pH = 7 at 26°C, and pH = 7.1 at 15°C. Notice that the signal at pH = 7 increases when the temperature is lowered. Lowering the temperature has no effect on the photosignal for pH = 2 (not shown). The access impedance was 40 kΩ, and the instrumental time constant was 1.77 μs.

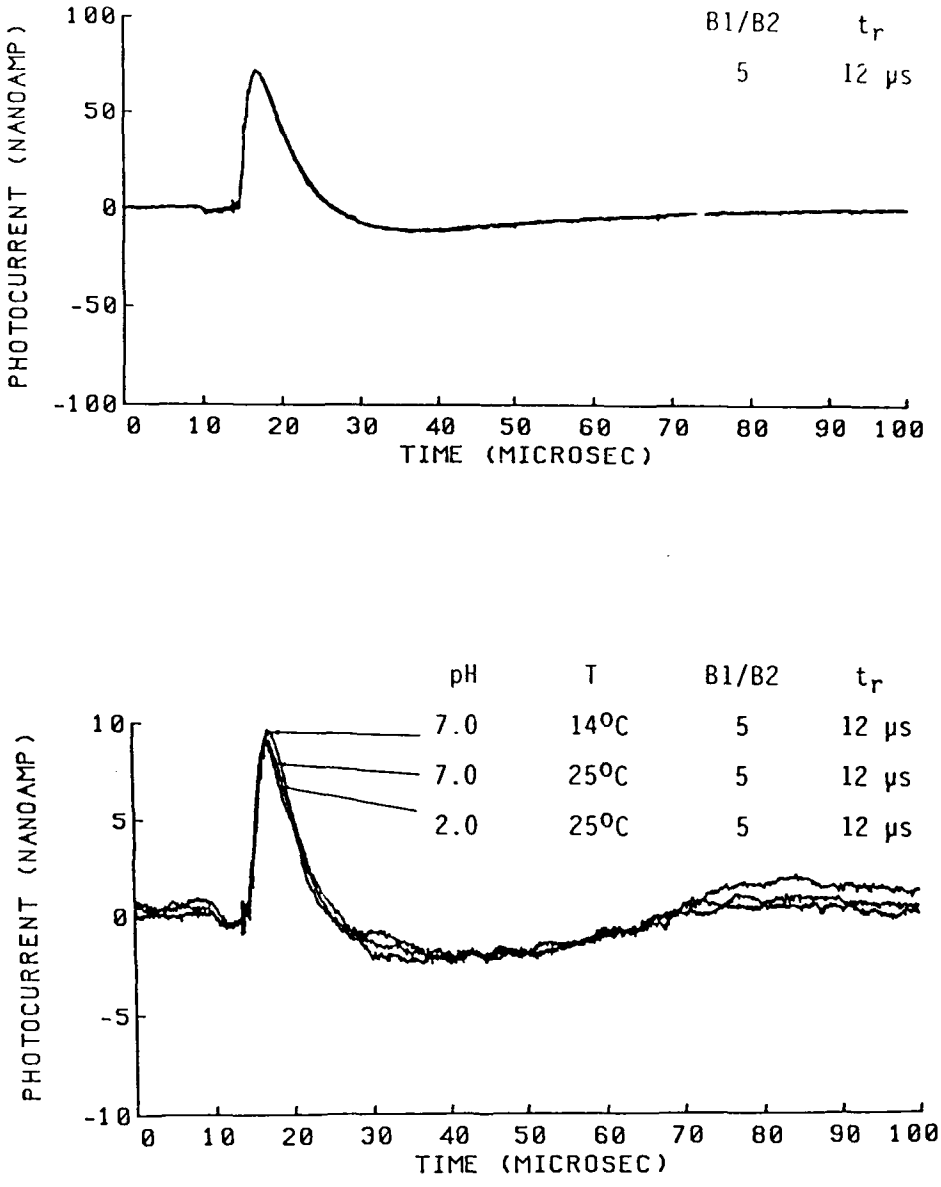

Fig. 4. (A) Effect of pH on the photoelectric signal from an aged multi-layered bR film. The aqueous phase contained 1.0 M KCl and 0.01 M L-histidine. The temperature was 26°C. There are four signals shown here for pH values of 2, 5, 7, and 8.5; the data are normalized to the signal for pH = 4. The data are completely superimposable after normalization. The access impedance was 40 kΩ, and the instrumental time constant 1.33 μs.

(B) Effect of pH and temperature on the photoelectric signal from an aged multi-layered bR film after stripping. The aqueous phase contained 1.0 M KCl and 0.01 M L-histidine. The data were taken in the following order: pH = 2 at 25°C, pH = 7 at 25°C, and pH = 7 at 14°C. Varying the pH and temperature have no effect on the photosignals. The access impedance was 40 kΩ, and the instrumental time constant was 1.77 μs.

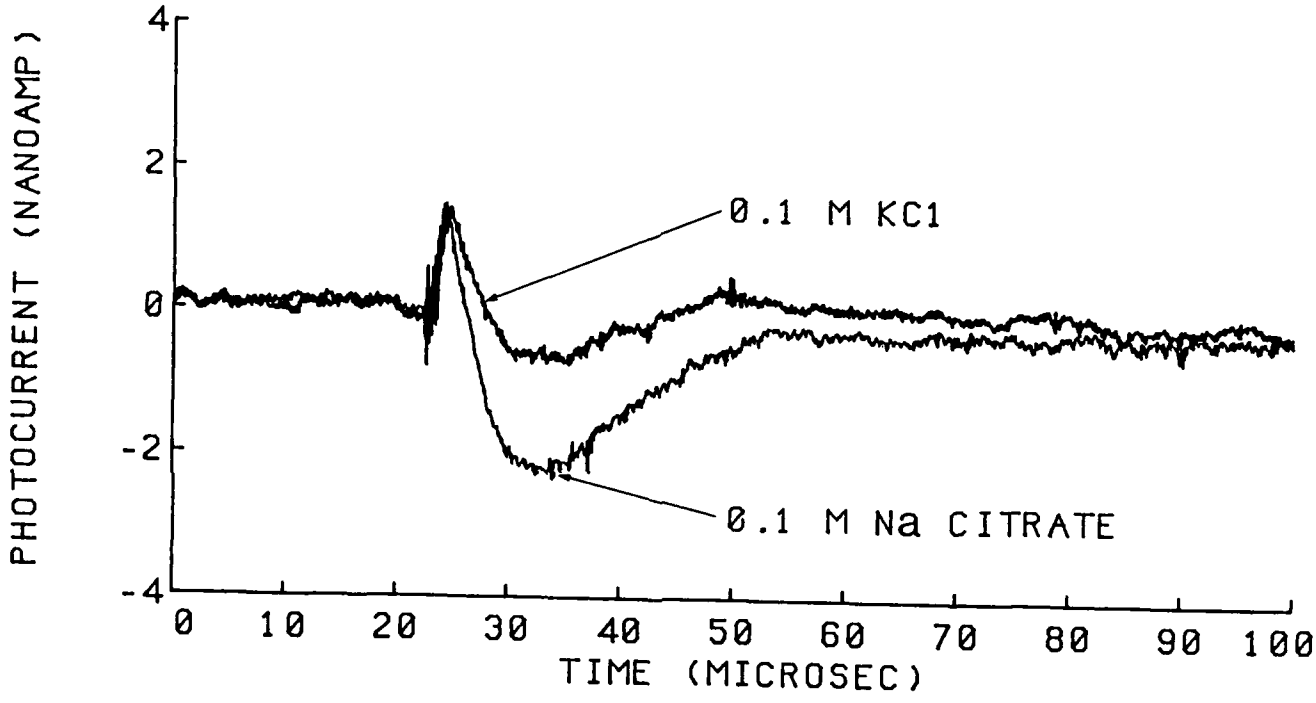

Fig. 5. Photoelectric signals from a Trissl-Montal type halorhodopsin thin film. The change of the photosignal is reversible when the aqueous solution is changed from KCl to sodium citrate and vice versa. The pH was 6 and the temperature was 25°C. The H1 component has a positive polarity and the H2 component has a negative polarity. The H1 component alone appears in a multi-layered dry film (not shown) and is not sensitive to the replacement of medium.

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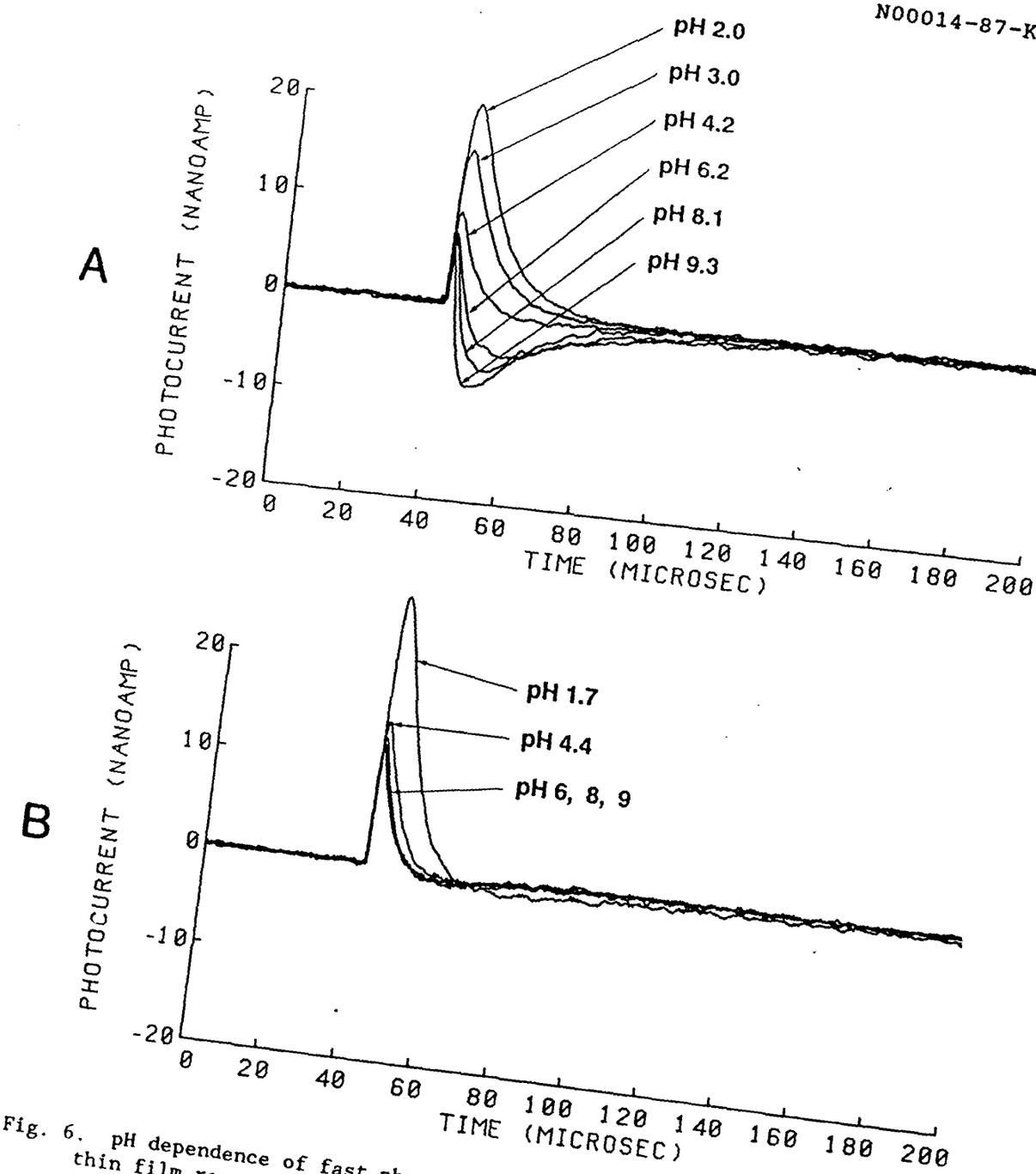

Fig. 6. pH dependence of fast photoelectric signals from a purple membrane thin film reconstituted from (A) the wild type bacteriorhodopsin and from (B) mutant bacteriorhodopsin with a point mutation at residue 212 (Aspartic acid → Asparagine). The data were both taken in 4 M NaCl solution at room temperature. (Unpublished data of Filbert H. Hong, Man Chang, Baofu Ni, Richard B. Needleman and Felix T. Hong)

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7. Hong, F. T., Pigment-Containing Membrane as a Prototype Biosensor, Proc. of the 1990 International Congress on Membranes and Membrane Processes, Chicago, IL August 20-24, 1990, Norman N. Li, Ed., pp. 215-217, North American Membrane Society, European Society of Membrane Science and Technology and Membrane Society of Japan (1990).

8. Michaile, S., Duschl, A., Lanyi, J. K., and Hong, F. T., Chloride Ion Modulation of the Fast Photoelectric Signal in Halorhodopsin Thin Films, in Proceedings of the 12th Annual International Conference of IEEE Engineering in Medicine and Biology Society, Philadelphia, PA, November 1-4, 1990, B. Oranal and P. C. Pedersen, Eds., pp. 1721-1723, Institute of Electrical and Electronic Engineers, Inc. Washington, DC (1990).

D. POSITION PAPERS IN GOVERNMENT PANEL REPORTS:

1. Hong, F. T., Bacteriorhodopsin as a Bioelectronic Material, National Science Foundation Biomolecular Materials Workshop Report, Washington, DC, October 10-12, 1990.

2. Hong, F. T., Learning from Nature by "Reverse Engineering" Photobiological Membranes, National Science Foundation Biomolecular Materials Workshop Report, Washington, DC, October 10-12, 1990.

3. Hong, F. T., Processing of Molecular Sensors and Bioelectronic Devices, in National Science Foundation Workshop on Submicron Particles, Washington, DC, November 19-20, 1990.

E. PUBLISHED ABSTRACTS:

1. Hong, F. T., Electrochemical Evaluation of Various Membrane Reconstitution Techniques, 13th Annual Northeast Bioengineering Conference, Abstr. 20.4, Philadelphia, PA, March 12-13 (1987).

2. Hong, F. T., Site-Specific Kinetics of Light-Induced Rapid Proton Movements Across A Bacteriorhodopsin Membrane and its Membrane-Water Interfaces, in Bioelectrochemistry Symposium, 193rd American Chemical Society National Meeting, Abstr. No. COLL 0112, Denver, CO, April 5-10 (1987).

3. Hong, F. T., Light-Induced Interfacial Charge Transfer in Biomembranes, 171st Electrochemical Society Meeting, Abstr. No. 385, Philadelphia, PA, May 10-15 (1987).

4. Hong, F. T., Interfacial Phenomena in Pigment-Containing Biomembranes, in Symposium on Interfacial Phenomena in Biotechnology and Materials Processing, 18th Annual Meeting of the Fine Particle Society, Boston, MA, August 3-7 (1987).

5. Hong, F. T., Relevance of Light-Induced Charge Displacements in Molecular Electronics: Design Principle at the Supramolecular Level, plenary lecture in Symposium on Molecular Electronic and Biocomputers, Budapest, Hungary, August 24-27, J. Mol. Electronics 3:28 (1987).

6. Hong, F. T., A Bioelectrochemical Study of the Bacteriorhodopsin Membrane System, in 9th International Symposium on Bioelectrochemistry and Bioenergetics, Szeged, Hungary, September 1-5 (1987).

7. Hong, F. T., Effects and Roles of Internal Electric Fields in Pigment-Containing Biomembranes, in 9th Annual Conference of the IEEE Engineering in Medicine and Biology Society, Boston, MA, November 13-16 (1987).

8. Hong, F. T., An Electrochemical Approach to the Design of Membrane-Based Molecular Optoelectronic Devices, in Symposium on Molecular Electronics - Biosensors and Biocomputers, 19th Annual Meeting of the Fine Particle Society, Abstr. No. WedAM-6, Santa Clara, CA, July 19-22 (1988).

9. Hong, F. T,, Bioelectrochemical Techniques in Molecular Optoelectronics, in XIIth Jena Symposium on Biophysical Chemistry: Trends in Bioelectrochemistry of Biopolymers and Membranes, Weimar, East Germany, September 19-24 (1988).

10. Hong, F. T., Control of Electric Signals in a Thin-Film-Based Molecular Optoelectronic System, in 10th Annual International Conference of IEEE Engineering in Medicine and Biology Society, New Orleans, LA, November 4-7 (1988).

11. Hong, F. T. Electrochemical Studies of Retinal Protein-Containing Membranes, in ONR Membrane Electrochemistry Program Investigators' Meeting, Elkridge, MD, November 27-29 (1988).

12. Hong, F. T., Electrochemical Studies of Retinal Protein-Containing Membranes, in Annual Meeting of the American Institute of Chemical Engineers, Washington, DC, November 28-30 (1988).

13. Hong, F. T., Electrochemical Approach to the Design of Bioelectronic

Devices, in Second International Symposium on Bioelectronic and Molecular Electronic Devices, Fujiyoshida, Japan, December 12-14 (1988).

14. Michaile, S., Duschl, A., Lanyi, J., and Hong, F. T., Fast Photoelectrical Signals From Reconstituted Halorhodopsin Membranes, 33rd Annual Meeting of the Biophysical Society, Cincinnati, OH, February 12-16, 1989, Biophys. J. 55: 387a, Tu-Pos363 (1989).

15. Hong, F. T., Physicochemical Processes Relevant to the Design of Biomolecular Optoelectronic Devices, in Symposium on Molecular and Biomolecular Electronics, Divisions of Biological, Chemical, High Polymer Physics, American Physical Society, St. Louis, MO, March 20 (1989).

16. Hong, F. T., Site-Specific Kinetics of Light-Induced Vectorial Proton Transport in Reconstituted Bacteriorhodopsin Membranes, Symposium on Electrical Double Layer and Ionic Processes in Biology, 175th Electrochemical Society Meeting, Los Angeles, CA, May 7-12 (1989).

17. Hong, F. T., Probing the Function of Engineered Proteins by Electrochemical Techniques, in Protein Engineering '89 Second International Conference, Kobe, Japan, 20-25 August (1989).

18. Hong, F. T., Does Mother Nature Utilize a Common Design in Photoactive Transport and Sensor Proteins?, plenary lecture in Second Symposium on Molecular Electronic and Biocomputers, Moscow, U.S.S.R., September 12-17 (1989).

19. Michaile, S., and Hong, F. T., Signal Modulation via Interfacial Processes in Molecular Optoelectronic Devices, in 11th Annual International Conference of IEEE Engineering in Medicine and Biology Society, Seattle, WA, November 9-12 (1989).

20. Hong, F. T., Electrochemical Analysis of Electronic Processes in Photobiological Systems, International Workshop on Progress towards Molecular Scale Electronics, Durham, UK, March 26-28 (1990).

21. Hong, F. T., Electrochemical Analysis of Photosensitive Biomembranes: Nature's Design Principles of Molecular Optoelectronic Devices, One Day Symposium on Electrochemical Systems and Applications, Detroit Section, Electrochemical Society, Bloomfield Hills, MI, April 19 (1990).

22. Hong, F. T., Bacteriorhodopsin as an Intelligent Material, International Symposium on "Biomedical Engineering in the 21st Century", Taipei, Taiwan, May 24-25 (1990).

23. Hong, F. T., Molecular Electronics: a New Horizon in Biomedical Engineering, International Symposium on "Biomedical Engineering in the 21st Century", Taipei, Taiwan, May 24-25 (1990).

24. Hong, F. T., Pigment-Containing Membrane as a Prototype Biosensor, 1990 International Congress on Membranes and Membrane Processes, Chicago, IL

August 20-24 (1990).

25. Hong, F. T., Electrical Processes in Photobiological Membranes, in 2nd All-Union Conference on Electronics of Organic Matter (ELORMA'90), Dombaj, USSR, September 20-25 (1990).

26. Hong, F. T., Retinal Proteins as Intelligent Materials, in NSF Workshop on Biomolecular Materials, Washington, DC, October 10-12 (1990).

27. Hong, F. T., Reverse Engineering of Photobiological Membranes, NASA Workshop on Aerospace Applications of Bionics, Charleston, SC, November 12-14, 1990.

28. Hong, F. T., Processing of Molecular Sensors and Bioelectronic Devices, NSF Workshop on Submicron Particles, Washington, DC, November 19-20 (1991).

29. Hong, F. T., Retinal Proteins as Intelligent Materials, in NATO Advanced Research Workshop on Coherent and Emergent Phenomena in Biomolecular Systems, Tucson, Arizona, January 15-19 (1991).

30. Hong, F. T., Do Biomolecules Process Information Differently Than Synthetic Organic Molecule?, Plenary Lecture in International Workshop and Initial Meeting of the International Society for Molecular Electronics and Biocomputing, Balatonszeplak, Hungary, 26-29 May, 1991.

31. Hong, F. T., Bacteriorhodopsin as an Intelligent Material, in Symposium on Molecular and Biomolecular Electronics, Fall 1991 American Chemical Society National Meeting, New York, NY, August 25-30, 1991.

32. Hong, F. H., Chang, M., Ni, B., R., and Hong, F. T., Modifying the photoelectric behabior of bacteriorhodpsin by site-directed mutagenesis, in 2nd International Conference on Molecular Electronics Science and Technology, St. Thomas, US Virgin Islands, December 15-19, 1991.

F. OTHER ACTIVITIES:

1. Invited Visiting Scientist, Institute of Physical and Chemical Research (RIKEN), Wako, Saitama, Japan, February 23 - March 2, 1987.

2. Organizer and Symposium Chairman, Symposium on Molecular Electronics - Biosensors and Biocomputers, 19th Annual Meeting of the Fine Particle Society, July 19-22, 1988, Santa Clara, CA.

3. Organizer and Chairman of the Molecular Electronics Track, IEEE Engineering in Medicine and Biology Society 10th Annual International Conference, New Orleans, LA, November 4-7, 1988.

4. Editor, Molecular Electronics: Biosensors and Biocomputers, 454 pp., Plenum Press, New York (1989).

5. Invited Visiting Scientist, USSR Academy of Sciences, Moscow and Leningrad, USSR, September 18-28, 1989.

6. Organizer and Chairman of the Molecular Electronics Track, IEEE Engineering in Medicine and Biology Society 11th Annual International Conference, Seattle, WA, November 8-12, 1989.

7. Invited Visiting Scientist, Southeast University, Nanjing, China, May 30-June 5, 1990.

8. Invited Visiting Scientist, Uzbek Academy of Sciences, Tashkent, Uzbek SSR, September 14-19, 1990.

9. Panelist, NSF Workshop on Biomolecular Materials, October 10-12, 1990, Washington, DC.

10. Organizer and Chairman of the Molecular Electronics Track, IEEE Engineering in Medicine and Biology Society 12th Annual International Conference, Philadelphia, PA, November 1-4, 1990.

11. Panelist, NASA Workshop on Aerospace Applications of Bionics, November 12-14, 1990, Charleston, SC.

12. Panelist, NSF Workshop on Fine Submicron Particles, November 19-20, 1990, Washington, DC.

13. Organizer and Chairman of the Molecular Electronics Track, IEEE Engineering in Medicine and Biology Society 13th Annual International Conference, Orlando, FL, October 31 - November 3, 1991.

14. Organizer and Chairman of the Symposium on Molecular Electronics, First World Congress for Electricity and Magnetism in Biology and Medicine. Orlando, FL, June 14-19, 1992.

15. Editorial Board Member, Advanced Materials for Optics and Electronics, starting 1992.

16. Editorial Board Member, Bioelectrochemistry and Bioenergetics, starting 1992.

17. Editor, Special Issue on Molecular Electronics, IEEE/Engineering in Medicine and Biology Magazine, 1992.